MICROBIAL DIVERSITY IN YELLOWSTONE HOT SPRINGS

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INTRODUCTION

Yellowstone National Park is characterized by hundreds of hot springs with high microbial diversity. For many years scientists have conducted many studies in order to learn more about the diversity of bacteria and archaea living in these extreme habitats. These bacteria and archaea are extremophiles organisms that can live in lethal conditions to most known species. These hot springs are distinguished not only by the size or depth, but also for its physicochemical parameters such as temperature, solar radiation, pH, or water composition, establishing a symbiotic, commensal or parasitic typical and very crucial relationship in a microbial community. The most important parameters that determine what kind of community prevails in a hot spring are the temperature and composition of the water. (1,2)

Some of the most studied hot springs are White Creek, Octopus Spring, Rabbit Creek, Bat Pool, Mammoth Hot Spring, Spring Cistern, Grand Prismatic Spring, Little Obsidian Pool and Hot Creek.

METHODS

Currently, massive sequencing techniques are used for this kind of studies, such as pyrosequencing, focusing on operational taxonomic units (OTUs) for the 16s rRNA gene. This technique offers a high performance, accuracy and flexibility.(3)

SITE	T (ºC)	pH	Phylum/Other
White Creek	48-50	8.2	Cyanobacteria (*Mastigocladus* sp.), Chloroflexi
Chocolate Pots	52	6.2	Cyanobacteria, Chloroflexi, Rhodococcales
Mushroom Spring	60	8.2	Chloroflexi (Red), Cyanobacteria (Synechococcus sp.)
Fairy Geyser	36-38	9.1	Chloroflexi (Red)
Bath Lake Vista	56-57	6.2	Chloroflexi (Green), Gammaproteobacteria
Dragon Spring	68-72	3.1	Aquificales (*Hidrogenobaculum*)
100 Spring Plain	72-74	3.5	Aquificales (*Hidrogenobaculum*)
Mammoth Hot Spring	70-72	6.5	Aquificales (*Sulfurihydrogenibium*)
Calcite Spring	74-76	7.8	Aquificales (*Sulfurihydrogenibium*)
Octopus Spring	80-82	7.9	Aquificales (*Thermocrinis*)
Bechler Spring	80-82	7.8	Aquificales (*Thermocrinis*)
Crater Hills	76	2.6	Crenarchaeota (Sulfolobales)
Nymph Lake	88	4.0	Crenarchaeota (Sulfolobales)
Monarch Geyser	78-80	4.0	Crenarchaeota (Desulfurococcales, Thermoproteales)
Cistern Spring	78-80	4.4	Crenarchaeota (Desulfurococcales, Thermoproteales)
Joseph's Coat	80	6.1	Crenarchaeota (Desulfurococcales, Thermoproteales)
Washbum Spring	76	6.4	Crenarchaeota, Aquificales
100 Spring Plain	72	3.4	Crenarchaeota

Figure 1. *The three major habitat types sampled include phototrophic (green), filamentous communities (blue), and archaeal-dominated, elemental sulfur-rich (yellow)* (6)

BACTERIA RESULTS

Cyanobacteria:
It's the most prevalent phylum.
Synechococcus elongatus, Mastigocladus laminosus, Calothrix, Oscillatoria, Leptolyngbya.
45-60ºC and usually alkaline pH

Synechococcus the most thermotolerant of which delimits the thermal maximum for photosynthesis (70ºC) (4)

Green non-sulfur bacteria:
Chloroflexi (Chloroflexus and Roseiflexus)
60-80ºC usually alkaline pH and 36-70ºC

Sulfur-oxidizing bacteria:
Aquificae (Thermocrinis ruber, Sulfurihydrogenibium azorense, Aquifex, Hydrogenobacter, Hydrogenobaculum, Hydrogenothermus) and Deinococcus-Thermus (*Thermus aquaticus*)
70-90ºC (>150ºC) alkaline and acidic pH.

Phylum minority: Proteobacteria (*Chromatium* <58ºC), Thermotogales, Acidobacteria and Bacteroidetes/Chlorobi (*Chlorobium* <54º)

ARCHAEA RESULTS

Crenachaeota:
It's the most abundant phylum.
Sulfolobus acidocaldarius (Thermoprotei) is the species most often isolated (5) pH 2-3 ideal and >85ºC

Nitrosocaldus yellowstonii (unclassified Crenarchaeota) *and Thermosphaera aggregans* (Thermoprotei). Acid pH (>85ºC)

Pyrolobus fumarii (thermoprotei). Temperature maximun 113ºC

Thaumarchaeota:
Nitrosopumilus maritimus (Nitrosopumilales)
Alkaline and acidic pH, 10-60ºC

Euryarchaeota:
Methanoculleus, Methanomicrobia y *Methanogenium*, Archaeoglobales (Methanomicrobia).
Acidic pH (>75ºC)

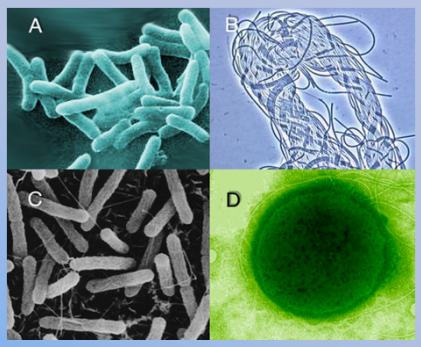


Figura 2*. Synechococcus elongatus (Cyanobacteria) (A), Roseiflexus castenholzii (Chroloflexi) (B), Thermocrinis ruber (Aquificae) (C), Thermococcus gammatolerans Crenarchaeota (D)*

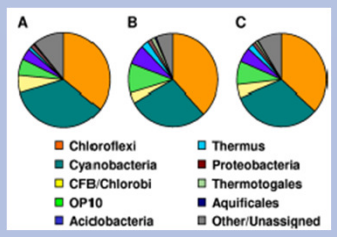
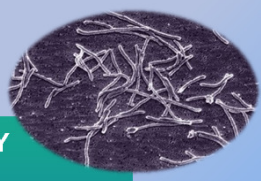


Figure 3. *Relative abundances of bacteria divisions in White Creek (A), Rabbit Creek (B) and the combined data set (C)* (7)



CURIOSITY

Thermus aquaticus (Deinococcus-Thermus) was discovered in 1969 in Lower Geyser Basin, Yellowstone. Currently, the heat-resistant enzyme *Taq DNA polymerase* is most commonly used in PCR (DNA amplification technique).

DISCUSSION AND CONCLUSIONS

Temperature is the most studied parameter, even when results obtained in studies where the water composition is analyzed are easier to explain and justify. Other influencing parameters but not taken so much into consideration are pH and the amount of radiation received. All these factors determine the diversity and richness of these microorganisms. The higher the temperature, the lower the diversity.

It is observed that temperature gradients are of wide ranges, which allows taxa to be distributed according to their most suitable temperature.

Some authors measure different temperature ranges for a single hot spring, but the important thing is that all authors agree that the most prevalent bacteria's phylum is Cyanobacteria which is located at lower temperatures, followed by Chloroflex and Aquificae at very high temperatures. These communities are in hot springs of different pH levels but always with alkaline or neutral waters.

As for the archaeal communities, these are less known and are found in hot springs with more acidic pH. The most predominant phylum is Crenachaeota, withstanding higher temperatures. It has been shown that archaea are more sensitive and vary more over time compared to bacteria. The study analyzed authors do not justify the root cause for these variations but presumably during the year, temperatures are causing ranging changes in physical parameters such as the fact of receiving higher radiation of light in summer and less in winter. The same applies to changes in water chemistry during the year and may lead to changes in pH and therefore to a restructuring of these communities, where competition among taxa would also be involved, and ultimately lead to changes in the number and type of microorganisms over time.

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•*Figure 2 taken from Bacmap Genome Atlas, American Society for Microbiology and from De la Torre Lab.*
•*Main photograph taken from Alterra: http://alterra.cc/en/Yellowstone_National_Park/.*

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